PHOTOSYNTHETIC ACTION SPECTRA OF MARINE ALGAE*

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INTRODUCTION

Englemann (1883, 1884) showed by an ingenious, though not strictly quantitative method (migration of motile bacteria to regions of higher oxygen concentration), that various algae displayed high photosynthetic activity in spectral regions in which light absorption was due mainly to pigments other than chlorophyll. From these now classic experiments it was concluded that, in addition to chlorophyll, other characteristic pigments of blue-green, red, and brown algae participate in photosynthesis.

Substantial proof of Engelmann's contention has come from the more extensive and exacting studies of this problem carried out in recent years. Dutton and Manning (1941) and Wassink and Kersten (1946) have shown that light absorbed by fucoxanthin, the major accessory pigment of diatoms, is utilized for photosynthesis with about the same efficiency as that absorbed by chlorophyll. However, the carotenoids of some other plants (which lack the fucoxanthin protein) appear to be relatively inactive; this situation has been reported for purple bacteria (French, 1937), *Chlorella* (Emerson and Lewis, 1943), and *Chroococcus* (Emerson and Lewis, 1942). In the latter blue-green alga the detailed study by these workers of quantum yield throughout the spectrum provided conclusive proof that another pigment, the water-soluble chromoprotein phycocyanin, is an efficient participant in photosynthesis.

The red algae, being in most cases macroscopic in form and marine in habitat, have not been studied by the quantitative methods applied to the aforementioned unicellular algae (*cf.*, however, the manometric studies of Emerson and Green, 1934, on *Gigartina*). Yet this group presents in the deep growing, bright red varieties striking examples of the occurrence of the chromoprotein phycoerythrin. This pigment, which may be accompanied in the chloroplasts of red algae by varying amounts of phycocyanin, has been studied by Kylin (1910), Boresch (1921), Lemberg (1928, 1930), Svedberg *et al.* (1928, 1929), and Tiselius (1930). Phycoerythrin absorbs strongly in the middle of the visible spectrum (490 to 570 mμ) and is thus almost complementary to chlorophyll in its absorption, rather than overlapping it as with carotenoids or phycocyanin. Hence, red algae are especially well adapted to distinguishing the roles of the two pig-

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ments by comparing photosynthesis at wave lengths chiefly absorbed by one or the other. Several investigators have made studies in this direction. Wurmser (1921) and Klugh (1930) employed the Osterhout-Haas phenol red indicator method for following CO_2 utilization in sea water. Wurmser used colored solutions to filter the sunlight; Klugh, Corning glass filters. Both found higher photosynthetic rates in green algae at the *ends* of the spectrum (blue and red light) while red algae showed an increased rate in the *middle* of the spectrum (green light). (Corrected to equal incident energies.)

Ehrke (1932), Schmidt (1937), and Montfort (1934, 1936) determined photosynthetic oxygen production by means of the familiar Winkler titration. All used Schott glass filters, which pass rather broad spectral regions. In green and brown algae a high photosynthetic rate was found in red light and in the blue end of the spectrum; the relative response in the latter region was usually higher in brown than in green algae. Red algae, on the other hand, almost always showed a maximum in the middle of the spectrum, green light being as much as 85 per cent more effective than red. Beyond indicating the possible participation of other pigments than chlorophyll, the results were not too conclusive because of the very broad spectral regions employed, which usually overlapped the absorption regions of more than one pigment. Other aspects of the experimental techniques used by some of these workers have been criticized by Emerson (1934, 1937).

More effective isolation of spectral regions was recently accomplished by Levring (1947) who also measured oxygen production by Winkler titration. A series of Schott glass filters, used either singly, in combination, or by a method of "differences," gave some 13 bands in the visible spectrum, of which 6 or 7 were fairly well separated. However, the transmission bands of these filters were still rather broad—often 100 to 200 mμ—and their "centers of gravity" were used in plotting the photosynthetic curves. This may seriously distort the results where the pigments have overlapping or steeply sloping absorption curves, different from those of the filters. This is often the case, as will be seen later in the present paper. However, within these limits and those imposed by working with different pieces of algae at different wave lengths, Levring's results were the most complete, up to their date, of relative photosynthetic rates in marine algae over the visible spectrum. Unfortunately he did not measure the absorption spectra of the algae used, but compared them with the published curves of Seybold and Weisweiler (1942). On the whole, just as in Englemann's work, photosynthetic rate in green and brown algae corresponded fairly closely with the absorption curves of chlorophyll and carotenoid pigments; in red algae light absorbed by phycoerythrin was more effective, the photosynthetic rate in the middle of the spectrum being about double that near the ends.

At about the same time the present authors (Haxo and Blinks, 1946) made

a comparison of photosynthetic rates in *Ulva* and *Schizymenia*,[1] using monochromatic light (or narrow bands) isolated from a mercury arc, with a rapid polarographic method of measuring oxygen production. The results, corrected in terms of equal incident *quanta* of light, were as follows (the rate in red light being taken as 100 per cent):—

Relative Photosynthetic Rates

	435.8 mμ	546 mμ	620–660 mμ
	per cent	*per cent*	*per cent*
Ulva (green alga)	94	46	100
Schizymenia (red alga)	48 to 53	288 to 340	100

While in general agreement with earlier work, the result with the red alga was so very striking, and the difference between it and the green alga so much larger than previously found, that it seemed desirable to undertake a more detailed study of both absorption and "action" spectra in a large number of marine algae, with a view to assaying the relative roles of the several pigments present. This demanded the use of essentially monochromatic light, in narrow bands over the entire spectrum. Since it is technically difficult to isolate such bands in high intensity, a very sensitive, and preferably rapid, method of measuring photosynthesis was necessary in order to plot action spectra with the same tissue over a wide spectral range in reasonable lengths of time. The polarographic method just mentioned appeared to provide this procedure, at least for *relative* action spectra (it is more difficult to make it absolute).

The results are presented in terms of graphs showing both absorption and photosynthetic action spectra for a number of red algae, and one representative each of the green and brown algae for comparison. It is felt that these supply the most detailed information yet available for absorption and action spectra in marine algae. It seems hardly necessary to emphasize at this time the value of action spectra in physiological analysis. These have had outstanding success in a number of well known cases, such as the identification of Warburg's respiratory enzyme and the implication of nucleoproteins as sensitizers in some ultraviolet effects on cells. The effectiveness of different spectral regions in inducing a given photochemical reaction is of course dependent first upon the absorption spectrum of the substance concerned; hence knowledge of the action spectrum of a reaction may lead to identification of the sensitizer. Thus, in green plants (*cf.* Hoover, 1937) the action spectrum for photosynthesis agrees fairly well with absorption in regions where chlorophyll is the only light absorber. But in regions such as the blue end of the spectrum,

[1] The thallus absorption curves made in conjunction with these rate measurements agree well with our later determinations presented in Figs. 8 and 21.

where other pigments absorb as well, the degree of correspondence between the activity and the absorption curves may indicate the extent to which the carotenoid pigments act as inert filters, or as sensitizers for the reaction. If all the pigments are active, then there should be a close correspondence. Both the shapes and the absolute values of the curves are of diagnostic use in this respect. It will be seen that the red algae, particularly, yield interesting examples of such correspondences and divergences.

Apparatus and Methods

Oxygen Determination.—

The studies were made possible by the voltametric or polarographic method of following photosynthetic oxygen production. This is a development of the work of Vitek (1935), who showed that the dropping mercury cathode, polarized in the region of 0.3 to 0.7 volt, could be used to measure the oxygen concentration of aqueous solutions. Baumberger (1938) applied this to physiology, and shortly thereafter it was used for photosynthetic measurements (Blinks and Skow, 1938; Petering and Daniels, 1938). Blinks and Skow also modified the cathode, first employing a stationary pool of mercury, and finally a bright platinum electrode in direct contact with the tissue. This greatly reduced diffusion distance and increased the speed of response making it possible to follow many details of the induction period during the first moments of illumination. When combined with a string galvanometer or cathode ray oscillograph (Blinks and Lewis, 1946), it was possible to record responses to single brief flashes of light. The polarized platinum cathode has also been employed in a revolving form by Kolthoff and Laitinen (1940), and in a microform by Davies and Brink (1942).

The principle of all these is the same: the cathode attracts positive ions but becomes rapidly polarized unless the ions can be discharged. The only ion of most solutions which can be discharged at this potential is the hydrogen ion, which can combine with oxygen at the electrode to form H_2O_2 (or water, at a higher potential). To the extent that hydrogen ions are discharged, a current can flow; this current will depend upon the oxygen which can diffuse to the electrode, hence, upon the oxygen concentration of the solution. The electrode can be calibrated by bubbling known mixtures of oxygen and nitrogen through the solution; the current is a practically linear function of the oxygen tension.

In closed vessels of solution, the dissolved oxygen is consumed by the electrode reaction itself, so that the current drifts slowly downward. If a respiring tissue is also present, the downward drift is faster; or if oxygen is produced photosynthetically, the current may conversely increase. The difference between the downward and upward slopes is then a measure of photosynthesis. This becomes a very sensitive method if small volumes of fluid are used, the minimum volume being reached with a glass plate placed directly over the tissue. Some earlier records of induction effects were taken with this arrangement.

A "steady state" modification of this arrangement was employed in the present study, the tissue being in direct contact with the electrode as before, but immersed

in a large volume of solution which can be stirred, flowed, or aerated. The oxygen in the external solution must diffuse across the tissue to reach the electrode, and in doing so is partly used up by tissue respiration. With this arrangement, therefore, the current flow is less than with a bare electrode (or a dead tissue, or an equivalent thickness of cellophane). The equilibrium value in the dark is taken as the base line. Upon illumination the tissue produces oxygen, which diffuses mostly toward the electrode (where the oxygen concentration is kept at a very low level because of combination with H ions at the electrode). The current increases, and the amount of the

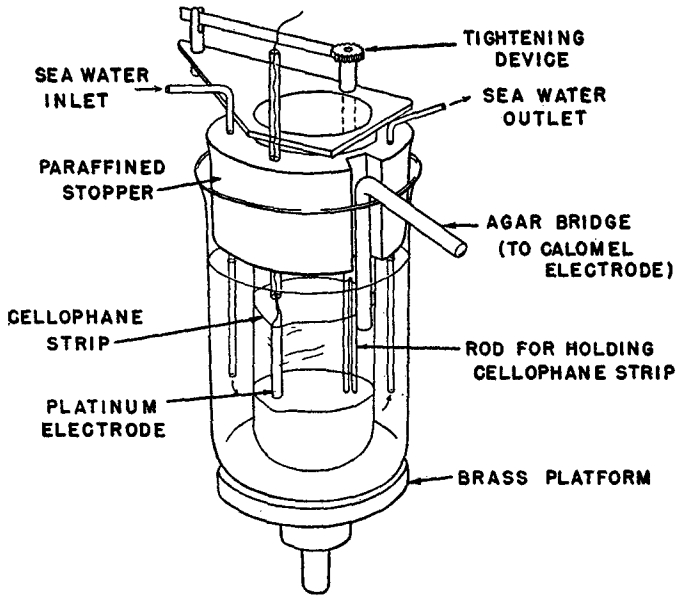

FIG. 1. Diagram of the reaction vessel. The platinum electrode is cemented to the test tube, and over it is mounted the algal tissue, which is held in place by a permeable cellophane strip (tightened by slotted rod at rear). Sea water in the beaker may be flowed through continuously, or aerated.

increase is taken as a measure of the photosynthetic oxygen production. (This increase is essentially linear when plotted against light intensity, as pointed out later.)

The actual arrangement, due to Dr. Charlton M. Lewis, is shown in Fig. 1. A bright platinum electrode (5 × 20 mm., and slightly curved) is cemented to a test tube (about 30 mm. diameter), the platinum lead wire being sealed through the tube or well varnished along its surface. The tissue is placed against the platinum and held tightly against it by a band of water-permeable cellophane, which is kept taut by means of a slotted rod at the back of the tube. The whole assembly is immersed in a larger vessel of sea water which may be aerated or renewed by flowing, assuring a constant supply of CO_2, nutrients, and oxygen. The circuit is completed through a sea water–agar salt bridge to a calomel electrode as anode.

The electrical circuit is shown in Fig. 2. A battery with potential divider P_1 supplies 0.5 volt to the system (electrodes plus decade resistance R_1). Leads from the terminals of the latter go to the galvanometer, which is properly damped (by R_2). The sensitivity is controlled by the value of R_1, which is usually kept fixed during a given experiment, but can be changed by a fixed ratio. There is almost no limit to the delicacy of the method if a very sensitive galvanometer is employed; we have used a Leeds and Northrup Type R, but this maker's wall galvanometer is usually adequate.

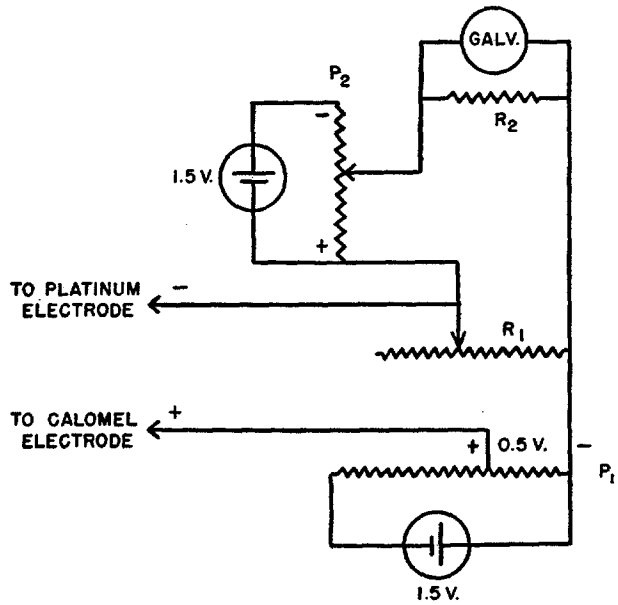

FIG. 2. Electrical circuit. A dry cell supplies current to the potential divider P off which is tapped 0.5 volt. This polarizes the platinum electrode cathodally, the circuit being completed through the calomel electrode. In series is the decade resistance R_1 across which is tapped the galvanometer (damped by R_2). A compensating E.M.F. can be introduced in this circuit by the second potentiometer P_2, to keep the galvanometer beam on scale.

No amplification is necessary unless rapid recording is desired with a string galvanometer or a cathode ray oscillograph. A compensating E.M.F. can be introduced into the galvanometer circuit by the potentiometer P_2 to keep the indicator spot on scale.

Monochromator.—

The demands upon this instrument were: (1) isolation of narrow spectral bands of good purity throughout the spectrum; (2) stable light output during individual measurements, and throughout the course of the action spectrum determination; (3) convenient equalization of light intensities throughout the spectrum.

These requirements were well met by the monochromator we constructed, after the designs of Dr. Charlton M. Lewis. It is of conventional pattern and differs from that described by French, Rabideau, and Holt (1947) largely in having achromatic lenses and a grating with good intensity throughout the first order spectrum, thereby simplifying many mechanical adjustments. A diagram of the arrangement is shown in Fig. 3. A 100 watt, clear-glass incandescent lamp was the light source (turned at

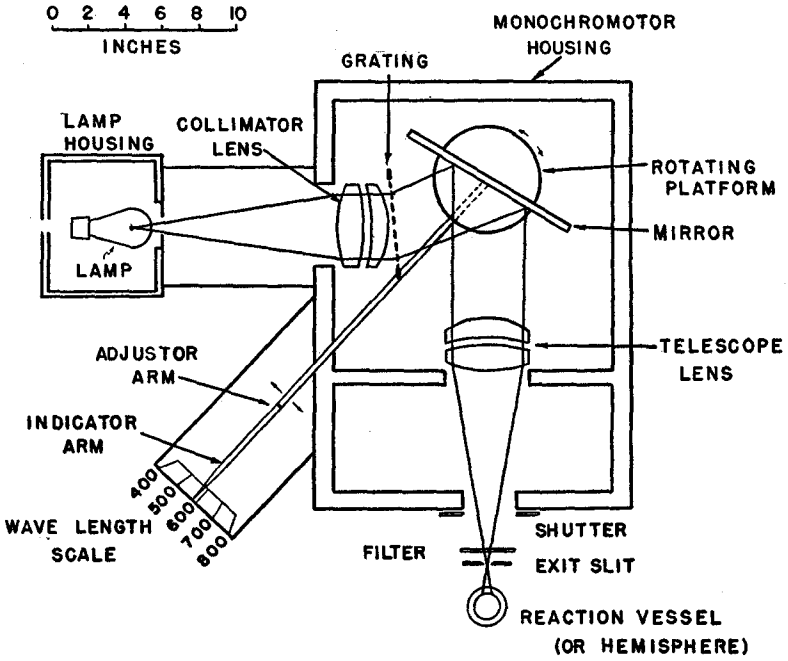

FIG. 3. Diagram of the monochromator. The light source is an incandescent lamp (with straight vertical filament) placed at the focus of the anastigmat collimator lens. A Woods replica grating disperses the light beam, which strikes the front-silvered plane mirror mounted on a rotating platform. Turning the attached arm swings the beam across the anastigmat telescope lens, which focuses the spectrum on the exit slit beyond which is placed the reaction vessel shown in Fig. 1. The indicator arm shows the wave length setting on a calibrated scale.

such an angle as to reduce reflections from the bulb surface to a minimum). The "straight" (helical) filament was at the focus of the collimating lens, in a vertical position; no entrance slit was used, though baffles were placed before the lamp in order to reduce stray light. The lenses were ground to order; they were 12.5 cm. in diameter with focus of about 30 cm. They were specified by the maker (Herron, of Pasadena) as being anastigmatic; however, they did show some coma. Horizontal coma was largely suppressed by vertical masks at the sides of the lenses; vertical coma, being in the plane of the slits, was not disturbing. Spectral dispersion was accom-

lished by a Woods replica (brightline) grating, of about 10 × 12 cm., ruled with 14,000 lines to the inch. This was prepared by Professor Woods himself, and was chosen from among several specimens as showing the highest "unilateral" first order intensity. It was set in the light path at a slight angle estimated as that giving the most intense spectrum. The dispersed beam from the grating next met a highly plane,

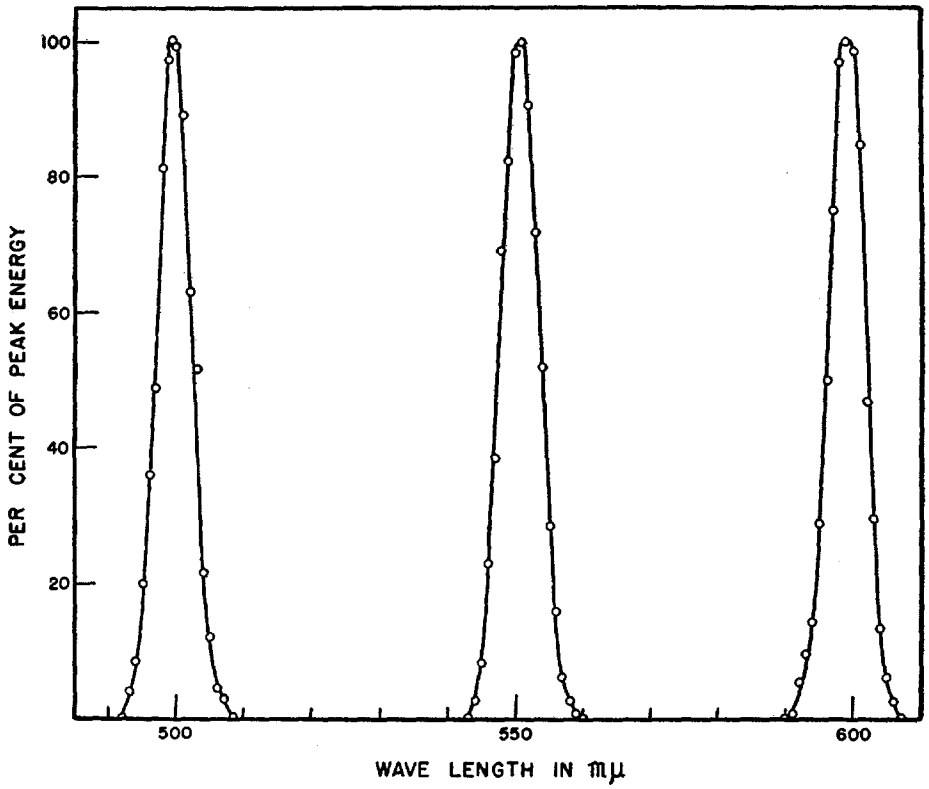

FIG. 4. Performance of monochromator. The relative distribution of the energy in spectral bands isolated from the monochromator with the exit slit at 0.75 mm. The wave length settings shown were 500, 550, and 600 mμ; other settings at 450 and 650 mμ gave similar curves.

front-silvered mirror, mounted on a precision-tooled platform. The latter could be rotated by a screw motion actuating an attached arm, thereby turning the reflected beam across the telescope lens, which brought the spectrum to a focus on the exit slit. A second, lighter pointer indicated the wave length setting on an illuminated scale. The latter was originally calibrated, and frequently checked against a Bausch and Lomb wave length spectrometer placed at the exit slit. The spectrometer was in turn calibrated against sodium and mercury lines. Dispersion of the monochromator

was 6.5 mμ per mm. at the exit slit. The adjusting screw of the latter was calibrated by means of measured plates or a wedge inserted in the jaws. A camera shutter was placed before the exit slit with attached cable release. A sliding carrier bore blue and red polished glass filters which could be used to reduce stray light when working at the ends of the spectrum. Infrared being refracted out of the field by the grating, it was found unnecessary to employ a heat filter.

Performance of the monochromator was tested first by visual inspection of the output through the spectrometer. When a 0.75 mm. exit slit was used, narrow bands 8 to 10 mμ were seen, with a minimum of stray light, at representative settings across the spectrum; when a 1.5 mm. slit was used, the bands averaged 15 mμ. Quantitative scanning of these bands was accomplished by substituting a photronic cell for the eyepiece of the spectrometer, with the analyzing slit set to throw a narrow band (5 or 2.5 mμ) upon the sensitive surface of the cell. The entrance slit of the spectrometer was set at 0.155 mm., and the beam was then caused to move across the exit slit by rotating the wave length drum of the spectrometer, while readings were taken of the photronic current. The relative distribution of energy with wave length was then plotted for a number of bands, allowance being made for the spectral sensitivity of the cell. The representative plots shown in Fig. 4 confirm the visual observation that the major part of the energy in each band fell within a narrow spectral zone. Thus, in the band centered at 500 mμ using a 2.5 mμ analyzing slit, 89 per cent of the energy fell within a width of 8 mμ, 95 per cent within 10 mμ, and 99.4 per cent within 15 mμ.

Relative light intensities were measured with an Eppley vacuum thermopile placed behind the monochromator exit slit. For absolute measurements the thermopile-galvanometer combination was calibrated against a standard lamp. In order to isolate bands of equal intensity throughout the spectrum at a fixed distance from the exit slit of the monochromator, the temperature of the incandescent lamp was varied by means of a "variac" and fixed transformers, the voltage across the lamp terminals being measured with a Weston thermojunction A. C. voltmeter. A calibration sheet was made for each lamp after insertion, showing the requisite voltages; these were rechecked at intervals between runs, although little change in output was found as the lamp aged. For the spectral region 480 to 750 mμ it was possible to obtain sufficient energy for a good response when the exit slit was set at 0.75 mm.; however, at wave lengths shorter than 480 mμ the lamps had to be burned at voltages up to 170 volts, and the slit width increased to 1.5 mm., resulting in a wider output band.

Absorption Spectra.—

In our preliminary experiments with *Ulva* and *Schizymenia* a Coleman spectrophotometer was used to measure absorption spectra, the fresh thalli being mounted in sea water in a cuvette, and the transmittance determined with a bleached thallus as blank. Such data are subject to uncertain errors due to scattering, etc., as pointed out by Mestre (1935); nevertheless, the curves did not differ greatly from those obtained later for these algae by the method described below.

Absorption spectra presented in this paper were obtained by a more satisfactory method, using the monochromator itself as a light source and a modified "Ulbricht

sphere" to take account of scattering.[2] The emergent beam from the exit slit was focused with prism and lens upon the surface of a horizontally placed photronic cell. A narrow strip (5 × 20 mm.) of the thallus was mounted directly on the glass cover of the cell, being kept moist and in place with a film of sea water and a coverslip. The proximity of tissue and light-sensitive surface assured the impingement of practically all transmitted and back-scattered light upon the photoelectric element. Front-scattered light was reflected back to the element from the "smoked" magnesia inner surface of the slotted hemisphere, which fitted over the photocell (Fig. 5). Reflection from the coverglass surface was determined by replacing the tissue with a blackened paper strip of equal size (prepared by coating black paper with lamp black suspended

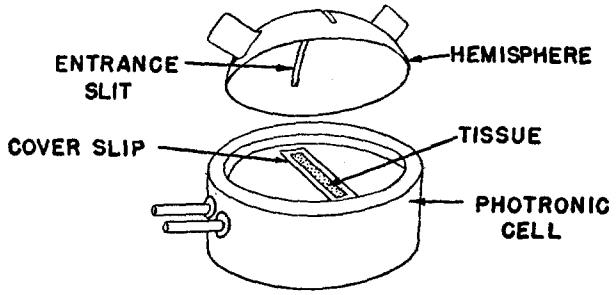

FIG. 5. Hemisphere for determining the absorption spectra of algae. The thallus strip is placed directly upon the glass cover of a photronic cell which catches transmitted and back-scattered light. Forward-scattered and reflected light is returned to the photronic surface by the smoked magnesia coating inside the hemisphere cover. Light from the monochromator is admitted through the slot, which also serves to restrict the entering beam so that it impinges on the tissue only.

in a detergent solution). Reflection from the tissue surface was in turn measured with the tissue placed over the blackened strip; since these readings were essentially the same as those obtained from the blackened strip alone, very low reflection (or scattering) is indicated at the tissue-water interface. Transmission was of course determined by the difference between the readings with and without tissue beneath the coverslip.

In practice, three readings were sufficient to calculate tissue absorption at any given wave length:

a = blank reading with sea water and coverslip alone;
b = reading with tissue;
c = reading with blackened strip (with or without tissue).

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[2] Much the same readings were obtained at several wave lengths (mercury lines) with the same tissue in a large Ulbricht sphere; our monochromator did not have enough energy to operate this with available photocells and amplifiers.

In terms of these readings the intensity of the incident light I_0 was calculated as

$$I_0 = a - c,$$

and that of the transmitted light I, as

$$I = b - c.$$

The per cent transmission then is

$$\text{per cent } T = \frac{b-c}{a-c} \times 100, \qquad \text{or } \frac{I}{I_0} \times 100.$$

Since the glass and tissue reflective losses are negligible, the tissue absorption A may be expressed as

$$\text{Per cent } A = 100 - \text{per cent } T.$$

It should be pointed out that some of the transmission (at all wave lengths) in monostromatous or thin algae such as *Ulva*, *Porphyra*, etc., is due to intercellular spaces (cellulose walls). This may amount to 10 per cent of the surface area. The plastids absorb more completely than the curves would indicate.

Whenever possible, spectral absorption curves were made either on the identical portion of thallus used previously for photosynthetic measurements, or on a directly adjacent piece cut from the same thallus. In the few instances where this could not be done, every effort was made to obtain pieces of like thickness and color.

Platinum Reflection.—

Since the polarographic method employed places the tissue in direct contact with a bright platinum electrode, there is opportunity for a second absorption of light; namely, that which passed through the thallus in one direction, was reflected from the platinum, and arrived at the thallus again. This is the same as having two layers of tissue, except for the light which may be absorbed by the platinum. The latter is by no means negligible, nor is it equal throughout the spectrum. Two methods of measuring it, either with the hemisphere just described, or by direct reflection to a photronic surface, yielded essentially the same results. For the platinum electrode employed, the reflection varied in a linear fashion from 57.5 per cent at 400 mμ to 73.5 per cent at 740 mμ. These values were used in correcting the activity curves for photosynthesis resulting from light absorption by the tissue on its second transit (see Experimental procedure below).

Pigment Extracts.—

The pigments were extracted from a few representative algae and their absorption spectra determined in order to estimate the distribution of the absorbed energy between the component pigments of the living thallus. We have, however, made no

attempt to reconstruct in detail the absorption conditions of the intact thallus on the basis of absorption by combined extracts with appropriate shifts in the absorption maxima. It must be admitted, in the case of the fat-soluble pigments, that the relative absorption by chlorophylls and carotenoids found in extracts may bear little relation to that obtaining in the cell; thus, this uncertainty prevails in appraising the role of carotenoids in photosynthesis. Fortunately, the absorption characteristics of the phycobilins are much the same in aqueous extracts and in the living thallus and a more direct comparison seems permissible. On the other hand, quantitative extraction and estimation of the water-soluble pigments are not as feasible.

The fat-soluble pigments of green and brown algae were readily extracted with 95 per cent methanol but most of the red algae were extracted better by exposing them first to dilute methanol, then by degrees to more concentrated methanol. After complete extraction the latter algae were red or pink, including species such as *Iridophycus* (*Iridaea*) *flaccidum* and *Porphyra perforata*, which were originally of a greenish or slate-grey color. (The phycobilins of the red algae are not extracted with methanol.)

Absorption curves of the total fat-soluble pigments were determined either directly in methanol or after transfer to ether. Carotenoids were separated by saponifying ether or petroleum ether solution with 10 per cent KOH in methanol, the resulting chlorophyllides being removed by repeated washings with water. Absorption by the carotenoid fraction was measured at the same dilution used for the total extract. Chlorophyll absorption was then calculated by difference.

Phycobilins extract from some red algae with ease, delicate forms such as *Antithamnion*, *Porphyra naiadum*, and *Delesseria* yielding their pigment rather readily into distilled water, or even when left unaerated in sea water. After several renewals of water the tissue may become pale green. Absorption spectra of the brilliantly fluorescing aqueous extracts were sometimes determined after filtration and centrifugation; in other cases the chromoproteins were further purified by precipitation with ammonium sulfate and redissolving in water or pH 7 phosphate buffer.

In other red algae more drastic procedures are necessary to extract the water-soluble pigments. *Porphyra nereocystis* and *Porphyrella* were ground in a mortar with quartz sand and water, the reddish brown solution being then filtered through paper and sintered glass, and finally centrifuged. The extracts were somewhat turbid and brownish rather than bright red; these characteristics persisted even after several precipitations with $(NH_4)_2SO_4$. Their absorption curves were not too different from those of the intact thallus, indicating the presence, perhaps in combination, of both phycobilins and fat-soluble pigments. Only after repeated shakings with ether could the latter be removed and a bright red solution obtained with yellow fluorescence. (The ether extracts contained both chlorophylls and carotenoids.) Transmittance measurements of such solutions still showed a disproportionately high absorption in the blue-violet, presumably due to absorption and scattering by protein impurities. In some cases, therefore, a bleached solution was used as the blank for spectrophotometry; this was prepared by adding a small amount of H_2O_2 and exposing to intense light, while cooling the sample in running water. (Bleached thalli may be prepared in the same manner.) A Beckman spectrophotometer was used in all determinations with extracted pigments, except in the case of *P. nereocystis*, where a Coleman instrument was employed.

Experimental Procedure.—

Flat marine algae were employed,[3] usually either one or two layers of cells thick; where possible, thin juvenile forms were used. Such algae have both optical and diffusion advantages. Light absorption is not as complete as in thicker forms, hence better absorption and action spectra can be traced; furthermore, diffusion of O_2, CO_2, nutrients, etc. is optimal, passing only one or two layers of cells. The short diffusion path makes the response to light rapid, the evolved oxygen needing to pass only the cell wall a few microns thick, to reach the platinum electrode which backs the tissue on one side.

After mounting the tissue on the electrode and placing the assembly in sea water, the tissue was usually given a period of light adaptation by exposure to a 25 watt incandescent lamp at a distance of 30 cm. for 20 minutes or more. Sometimes this exposure was made in the monochromator beam itself, using a widened slit, at a wave length of the highest photosynthetic effectiveness for the particular alga. This preliminary adaptation increased the stability of the tissue response to later monochromatic light exposures. After this treatment and at the low light intensities employed during the rate measurements, "induction effects" such as the "oxygen gush" (*cf.* Blinks and Skow, 1938) were not encountered.

After the adaptation period, the reaction vessel was carefully aligned so that the tissue was in the middle of the emergent monochromatic beam; the galvanometer sensitivity was adjusted (by the decade dial R_1) so that the maximal photosynthetic response occupied about $\frac{3}{4}$ of the scale (50 cm. long). The shutter was then closed, and the current allowed to reach its "dark" value, this requiring about 10 minutes. By means of the compensating E.M.F. (P_2), the galvanometer spot was brought to zero on the scale. Upon illumination the galvanometer reached a new, steady "light" value within $\frac{1}{2}$ to 2 minutes, provided that the outer sea water was flowing or aerated. The reading was then taken and the shutter at the exit slit closed. A new "dark" equilibrium was established at the electrode in $1\frac{1}{2}$ to 4 minutes, a more rapid response being obtained with thin thalli. The exposure sequence was repeated at least once for each wave length setting, the number of repetitions being determined by the reproducibility of the readings. The average difference ($\triangle$) between the dark and light readings was recorded as the measure of photosynthetic rate. The time course of a typical electrode response is shown in Fig. 6. Although the dark value was usually quite reproducible, necessitating very little adjustment of the zero, there was occasionally a tendency for it to drift, sometimes in an irregular manner and for no apparent reason. On the other hand, if the solution was not flowing or aerated, a regular upward creep in the dark value took place because of the accumulation of dissolved oxygen from photosynthesis.

The response ($\triangle$) to light was found to be almost strictly proportional to intensity

[3] The algae were freshly collected on the shores of Monterey Peninsula, California, and identifications made according to Smith, G. M., Marine Algae of the Monterey Peninsula California, Stanford University, 1944. They were usually employed for experimental purposes within 2 or 3 days after being brought into the laboratory (where they were maintained in running sea water and exposed to diffuse daylight.

within a range exceeding that employed in the determination of action spectra. For such determinations the incident intensity was about 5 to 10 ergs/sec./mm.^{2}; photosynthesis at this value was near "compensation" (*i.e.*, about equal to respiration) and below light saturation. In Fig. 7 the linearity of response at several wave lengths is shown for *Porphyra naiadum*. At higher intensities up to the maximum delivered by the monochromator with wide slits (110 erg/sec./mm.^{2}), the algae tested showed a falling off in rate. This was gradual for some algae and may have been due to an

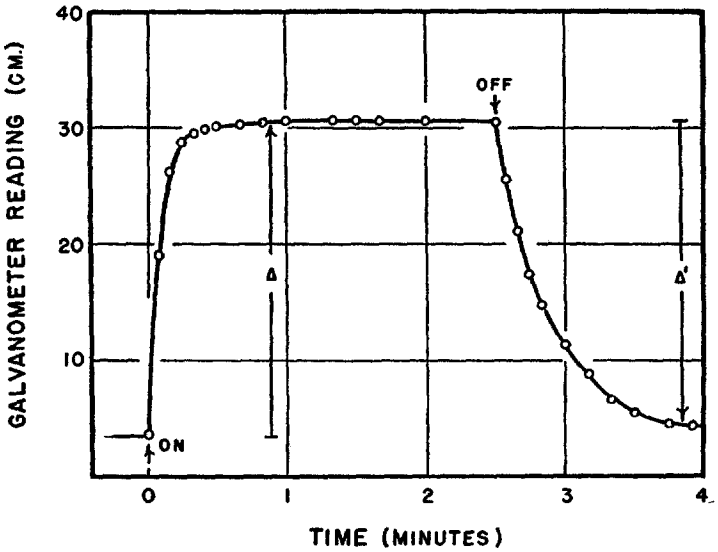

FIG. 6. Time course of galvanometer deflection in light and dark. The dark current is due to the arrival of oxygen from the solution across the tissue to the electrode. It has been brought approximately to zero by a compensating potential *in the galvanometer circuit*. (It still flows in the main circuit.) On illumination (indicated by arrow), oxygen is produced by the alga (in this case *Ulva*), the current increases at the electrode, and the reading increases to a new steady level within 1 minute. On darkening, it returns to its dark value within 2 minutes. The average difference in levels (Δ and Δ') is a measure of the photosynthetic rate.

inadequate CO_2 supply from the bicarbonate of the sea water. Some of the red algae tested, however, may actually reach or approach light saturation at the higher intensities (Fig. 19).

Readings were then taken at equal incident energy throughout the visible spectrum, usually in a systematic fashion at intervals of 10 mμ. More or less equivalent light and dark periods were employed in order to standardize the response. The complete action spectrum could be taken in this detail within 3 to 6 hours depending on the number of replicate readings made.[4] A temporal drift in magnitude of response

[4] For purposes of preliminary orientation or for demonstration, the spectral response of an alga can be scanned roughly in a few minutes merely by observing the course

sometimes occurred; this was allowed for by redetermining at intervals the response at some optimal reference point (*e.g.*, 560 mμ for red algae and 675 mμ for green and brown algae) and applying a percentage correction to the relative rates at other wave lengths. The sequence of the wave length settings had no effect upon the observed rates. The tissue remained in good condition on the electrode, and a second action spectrum could sometimes be taken on the same day, or on the next day, providing

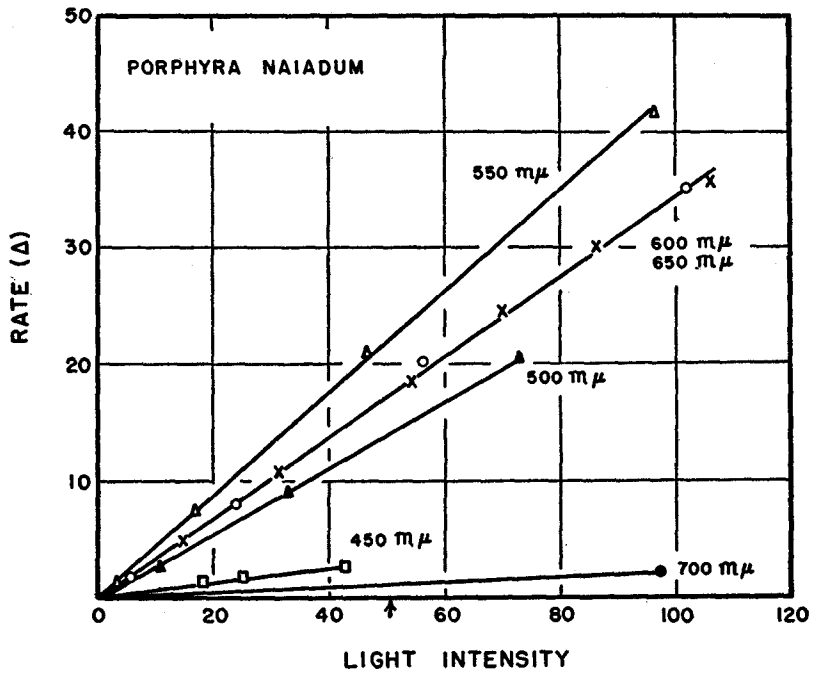

FIG. 7. Photosynthetic response (△) plotted against relative intensity of incident light. Although the slopes may vary with different wave lengths, each curve is rectilinear. Absolute intensities were not measured in this experiment; however, the intensity usually employed in the action spectra determinations (8 ergs/sec./mm.^{2}) would fall on the ordinate approximately at the arrow. Photosynthesis at this intensity was below saturation and above the compensation point.

sea water had remained flowing through the vessel. The absolute rate was sometimes lower on the 2nd day, but the relative action spectrum remained unchanged. In practice only freshly mounted tissues were used for critical work.

The raw data so obtained were subject to two corrections before plotting: (*a*) rates were recalculated in terms of equal incident *quanta* (rather than equal incident energy as measured). This raised the curves progressively toward the blue end of the spectrum; (*b*) allowance was made for the added photosynthesis due to light transmitted

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of the galvanometer spot when the shutter is left open and the wave length dial moved very slowly along the spectrum.

through the tissue, reflected by the platinum electrode, and absorbed on its second passage. The magnitude of this correction depends on two factors at any given wave length: the transmission of the tissue, and the per cent reflection of the platinum. The correction is greatest in regions of the spectrum *least* absorbed by the tissue. Thus, green light (540 mμ) may be absorbed only 25 per cent in its passage through one layer of *Ulva* (see Fig. 8); 75 per cent passes through, of which 65 per cent is in turn reflected by the platinum. Therefore, 49 per cent of the original light is returned to the tissue; 25 per cent of this is absorbed in the second transit, or about 12 per cent

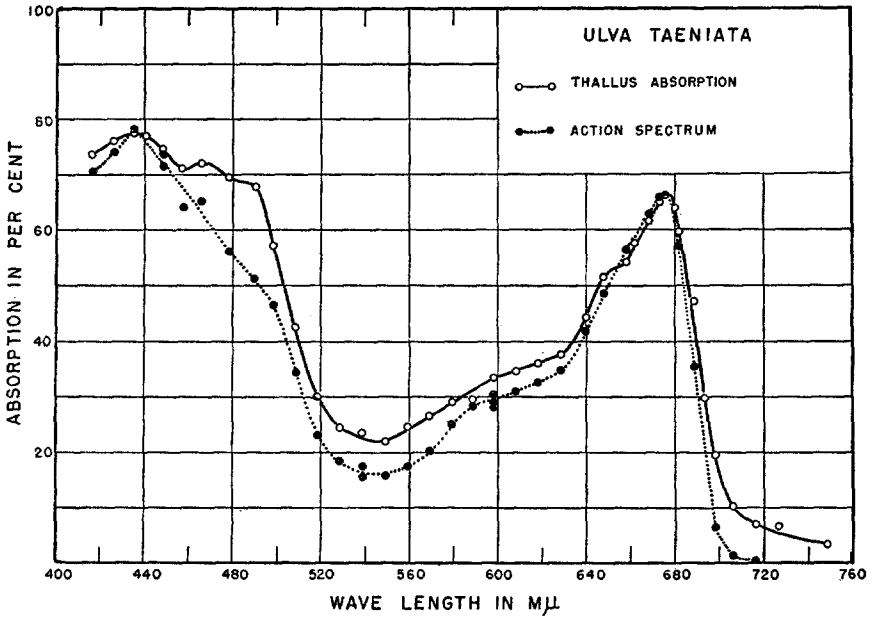

FIG. 8. Absorption spectrum of the green alga *Ulva taeniata* (taken in the apparatus shown in Fig. 5). The photosynthetic action spectrum, corrected to relative rates for equal incident quanta, has been made to coincide with the former at 675 mμ; the correspondence through the rest of the spectrum is then good. Deviations suggest inactive absorption.

of the original energy. The total absorption is 25 per cent + 12 per cent = 37 per cent, of which about a third is due to the reflected light.

On the other hand, the incident blue light (436 mμ) was almost 80 per cent absorbed on its first passage through this alga. 12 per cent of the original energy was reflected back, of which 80 per cent (or 10 per cent of the original energy) is again absorbed on the second passage. Total absorption: 80 per cent + 10 per cent = 90 per cent, of which only one-ninth is due to the second passage.

It seemed more generally useful, especially for comparison with other methods, to express *all* results, both absorption and action spectra, in terms of one light passage through the tissue, uncomplicated by the reflecting properties of the particular

platinum surface we used. Consequently, all observed readings have been revised downward by use of the correction factor: $(\frac{1}{1 + rT})$, where r is the reflection of the platinum (ranging from 0.575 to 0.725) and T the transmission of the tissue $(1 - A)$ at the given wave length (A = absorption factor).

RESULTS

Green Algae (Chlorophyta).—

The experimental curves, so derived and calculated, may now be considered. Partly to test the method, since the action spectra of green plants are best known, the green algae *Ulva* and *Monostroma* were studied. The former was the more stable experimental material; the results with *Ulva taeniata*, a thin, delicate species, are shown in Fig. 8. The open circles are the per cent absorption, plotted against wave length. The characteristic absorption peak (here 66 per cent) for chlorophyll appears at 675 mμ, absorption falling away rapidly toward 720 mμ and less rapidly toward 600 mμ, where a small, second maximum appears. The minimum (22 per cent) is at 550 mμ in the green, with a sharp rise of absorption again toward 500 mμ; it remains at 70 per cent or more to the limit of observation (420 mμ). The absorption between 400 and 500 mμ is of course due to carotenoids as well as chlorophylls, the latter with a peak at about 435 mμ (Fig. 9). This curve is in good agreement with most published ones for living green tissues; because of the thin thallus, *Ulva* shows more transmission in the green than most leaves.

Fig. 8 also shows the action spectrum of *Ulva*. In this curve the relative photosynthetic rate (corrected as described above) was arbitrarily made to coincide with the absorption at 675 mμ (the chlorophyll red maximum). This one point having been made to agree, it is seen that all the rest of the curve closely follows the absorption spectrum, including the second slight maximum in the orange (600 mμ), the minimum at 540 to 550 mμ, and the maximum at 435 mμ. (The exact agreement of the latter is no doubt fortuitous, since other examples show some deviation.) Qualitatively, action and absorption are in close agreement, indicating that most of the pigments are active sensitizers. Quantitatively, there are divergences: (*a*) in the region 700 to 740 mμ, noted previously and its significance discussed by Emerson and Lewis (1943) for *Chlorella*; (*b*) in the chlorophyll minimum around 540 mμ, which may be partly due to possible errors in the large reflection correction introduced here; and (*c*) in the region 450 to 510 mμ, where the action spectrum lies 10 to 15 units below absorption. We do not wish to emphasize this unduly, but the latter deviation is doubtless an expression of inactive absorption by carotenoids, as suggested by Emerson and Lewis for both *Chlorella* and *Chroococcus*. However, not all the carotenoids (either individually or collectively) would appear to be

inactive; otherwise the effectiveness spectrum could not be so high at 440 mμ, where *in extracts* carotenoids absorb almost as much light as does chlorophyll (Fig. 9).

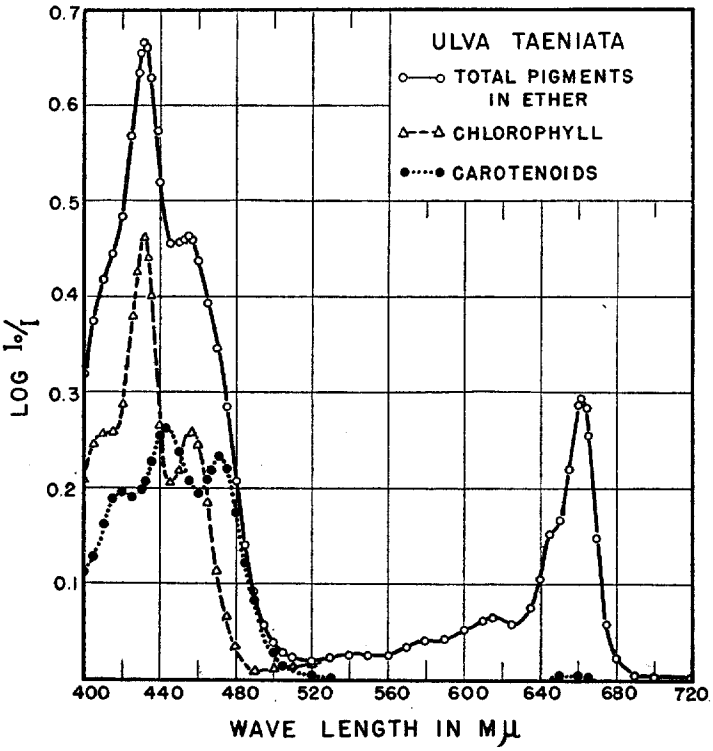

FIG. 9. Absorption spectra (in ether) of the fat-soluble pigments from *Ulva taeniata*. The carotenoids were measured at the concentration obtaining in the total pigment extract. The chlorophyll curve was calculated by difference.

Brown Algae (Phaeophyta).—

The characteristic color of brown algae and diatoms is apparently attributable to the physical state of the carotenoids (especially fucoxanthin) in the chloroplasts, rather than to an unusually high content of these pigments. Upon heating, the plastids change color from brown to green. Menke (1940), and in particular Wassink and Kersten (1946), attribute this spectral shift to the coagulation of a fucoxanthin protein, which in the living cell is responsible for the strong absorption between 500 to 560 mμ.

Menke's study of the color change in *Laminaria*, the only one available for Phaeophyta, was limited to a visual determination of the thallus absorption

bands. The extinction curves shown in Fig. 10 of this brown alga, before and after heat denaturation, are therefore of interest. Comparing the two curves it may be noted that the observed greening is accompanied by an over-all decrease in absorption from 500 to 570 mμ and its increase in the region 570 to 665 mμ. The wave length of minimum absorption has shifted from 600 mμ

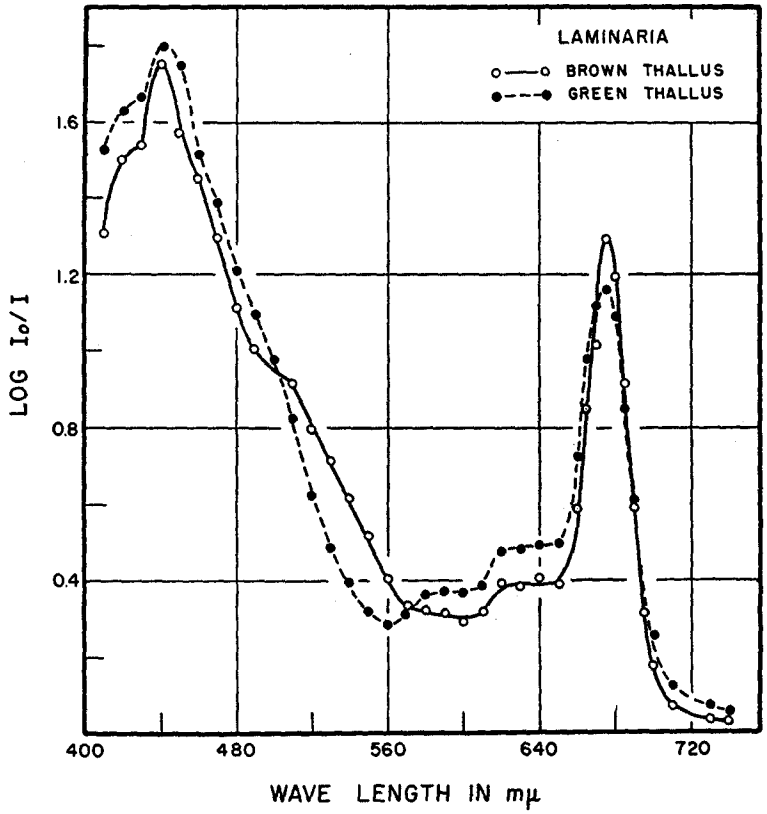

FIG. 10. Extinction curves of the brown alga *Laminaria* sp. The thallus was killed by dipping briefly in near boiling water, resulting in a color change from brown to green. The decrease in extinction shown by the green thallus in the region 500 to 560 mμ is presumably due to the release of fucoxanthin from its protein.

in the living thallus to 560 mμ in the denatured thallus. There is but little change in the absorption below 500 mμ, although the shoulder at this point has disappeared. The position of the chlorophyll maximum at 675 mμ has not shifted, but the absolute absorption has decreased slightly. Beyond 700 mμ the absorption (or opacity?) of the greened thallus has increased.

The available brown alga best suited for the measurement of photosynthetic

rates by our method was *Coilodesme californica*. The expanded portion of the thallus is a compressed sac which upon splitting provides a very thin delicate sheet, only a few cell layers thick. The absorption curve of *Coilodesme* shown in Fig. 11 is generally similar to that of *Laminaria*, although the over-all absorption is much less and the middle region is lower. (Another brown alga, *Ilea fascia*, was intermediate to these two in its absorption.) In comparison with *Ulva* are to be noted the shift in the absorption minimum to 600 mμ and the more rapid rise in absorption toward the blue-green due to fucoxanthin.

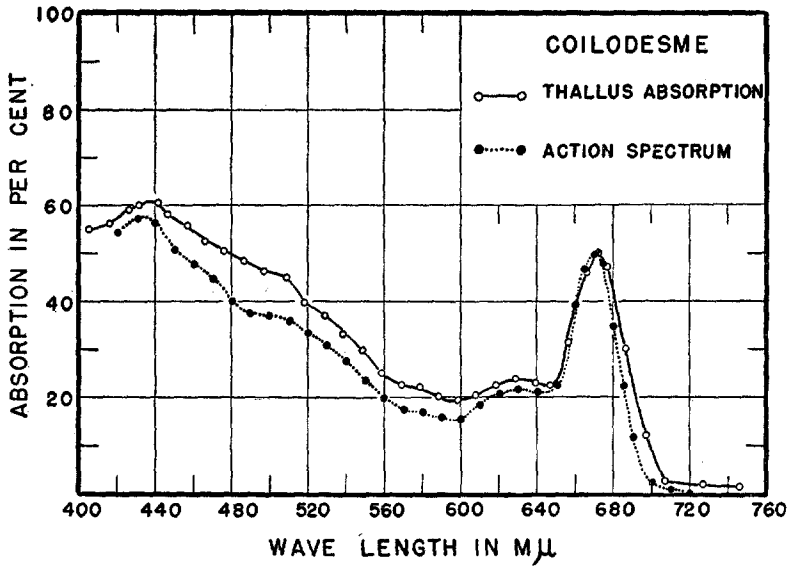

FIG. 11. Absorption and action spectra of the brown alga *Coilodesme californica*. The curves, having been made to coincide at 675 mμ, show only minor divergences elsewhere. The high activity in the region 500 to 560 mμ suggests the participation of fucoxanthin.

The steeper drop from 675 mμ toward shorter wave lengths with the minimum at 650 mμ is due to the absence of chlorophyll *b* in brown algae.

The action spectrum (again made to coincide with absorption at the chlorophyll peak in the far red) follows the absorption spectrum almost as well as in the case of *Ulva*. The deviation at 700 to 720 mμ, and the increasing divergence from 600 mμ down to about 460 mμ are to be noted. But at 435 mμ action is again almost as high as absorption. In the region 500 to 560 mμ where fucoxanthin absorption is presumably high, some inactive absorption is indicated; however, while fucoxanthin may not be quite as efficient as chlorophyll, much of the light absorbed by it is evidently utilized. The same may perhaps be said

of the other carotenoids present; otherwise photosynthesis could hardly be so high in the region 420 to 500 mμ where carotenoids account for some half of the light absorption (*in extracts, cf.* Fig. 12). No attempt was made to resolve the carotenoid pigment fraction and identify the components.

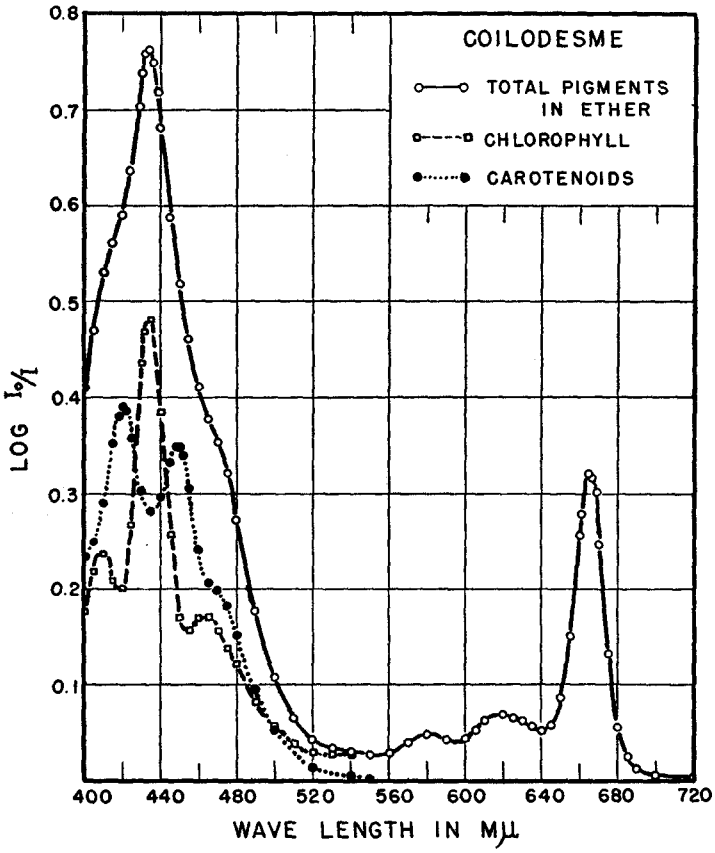

FIG. 12. Absorption spectra (in ether) of the fat-soluble pigments from *Coilodesme californica*. About half of the absorption in the blue end of the spectrum is due to carotenoids.[5]

[5] *Note Added in Proof.*—The absorption curves given in Fig. 12 possibly represent incorrectly the relative absorption by carotenoids and chlorophylls in *Coilodesme*, since the fucoxanthin content of the original extract could well have been altered during saponification of the chlorophylls. (At the time these determinations were made we were not aware that fucoxanthin, unlike other carotenoids, is decomposed by alcoholic potassium hydroxide, *cf.* Strain, 1949.) The anticipated correction is in the direction of stronger absorption by the carotenoid fraction, notably in the region 450 to 520 mμ.

Action spectra, uncorrected for back reflection, were also determined for *Laminaria* and *Ilea fascia*, both of which have rather thick thalli and show stronger absorption in the fucoxanthin region. The major features of their activity curves were like those of *Coilodesme*, although they showed higher activity in the middle of the spectrum (500 to 560 mμ). The action spectra of these three thalloid brown algae, then, support the conclusions of Dutton and Manning (1941) and Wassink and Kersten (1946), based on radiation of diatoms with a selected few spectral lines and bands, that fucoxanthin is an effective light absorber in photosynthesis, along with chlorophyll. However, in brown algae, as in diatoms, there is no evidence obtained to support Montfort's claim (1941) that light absorbed by fucoxanthin is *more* effectively utilized than that absorbed by chlorophyll.

Red Algae (Rhodophyta).—

We now turn to a very different situation in the red algae. Here, in addition to chlorophyll and carotenoids, there is present an abundance of phycoerythrin and usually, also, the related chromoprotein phycocyanin, in greater or less degree. The phycocyanin of red algae differs from that found in the blue-green algae and has absorption maxima at 553 and 615 mμ (Svedberg and Lewis, 1928).

(*Bangiales*).—A series of red algae in which there is a graded decrease in the ratio of phycoerythrin to phycocyanin consists of three species of *Porphyra*; *P. nereocystis*, *P. naiadum*, and *P. perforatum*. These are members of the primitive order Bangiales, with thin thalli one cell thick, abundant, and very well adapted to our method of measurement. *P. nereocystis* has the least phycocyanin; it is deep pink to dull purplish red in color. *P. naiadum* has large amounts of both phycoerythrin and phycocyanin, and is purplish red to deep purple. *P. perforata* has proportionately the least phycoerythrin and the most phycocyanin; it is steel-gray or slate-green in color, but does become brick red at the margins (carposporic areas) a few days before the release of reproductive cells.

Fig. 13 shows absorption and action spectra for *P. nereocystis*, the reddest and deepest growing member of the series. While characteristic chlorophyll absorption maxima are seen at 675 mμ and 440 mμ, just as in *Ulva* and *Coilodesme*, there are other maxima: at 495 mμ, 540 mμ (not so evident in this curve), and 565 mμ. These three are due to phycoerythrin. The absorption spectra of the fat-soluble pigments given in Fig. 14 show the relative absorption by chlorophylls and carotenoids in extracts and indicate that the minor hump in thallus absorption at 620 to 630 mμ, typical of all red algae tested, is at least *in part* due to chlorophyll *a*. The action spectrum (here plotted slightly below the absorption spectrum) is seen to parallel the phycoerythrin curve far more closely than it does that of the chlorophyll, thus identifying the former as the

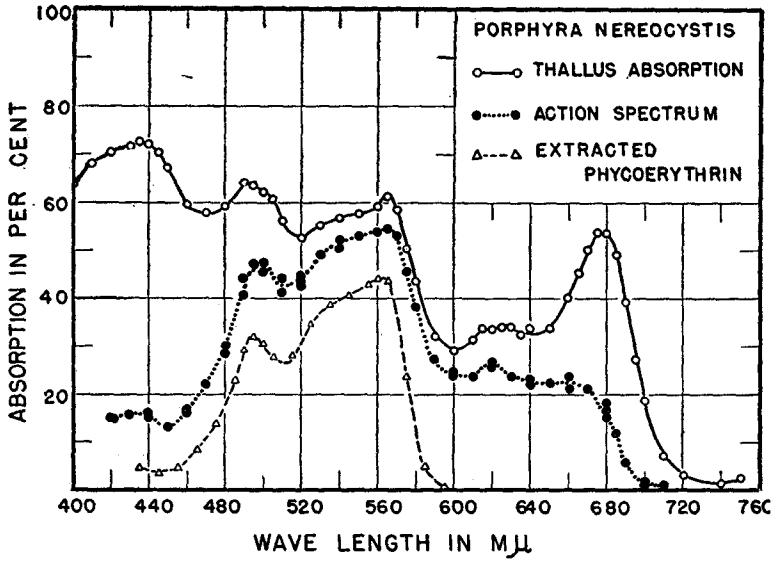

FIG. 13. Absorption and action spectra of the red alga *Porphyra nereocystis* (in which phycoerythrin is the principal phycobilin pigment). The action spectrum corresponds more closely to the water extract of phycoerythrin than to the absorption curves of chlorophylls and carotenoids (refer to Fig. 14).

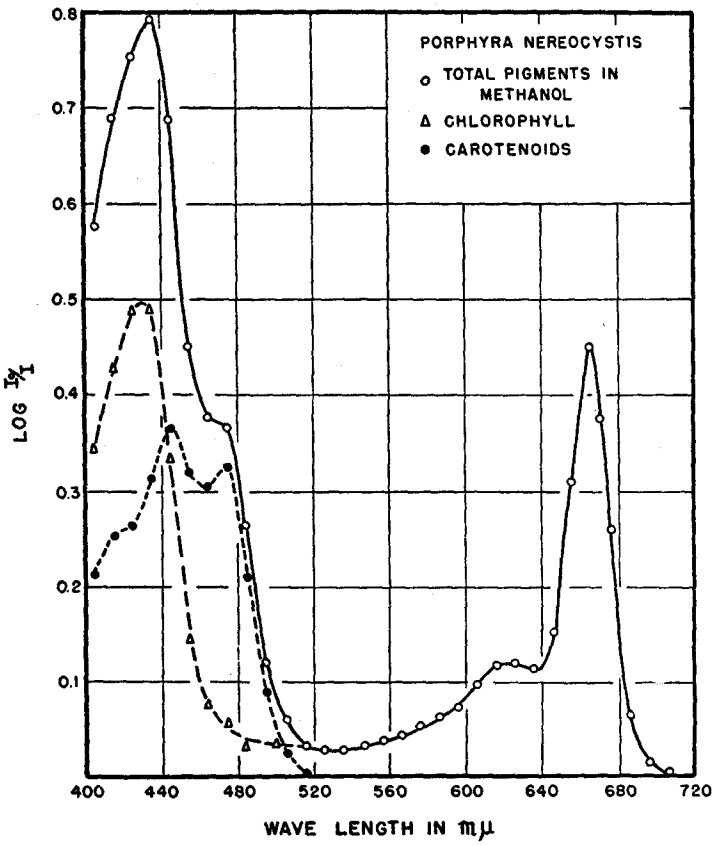

FIG. 14. Absorption spectra in methanol of the fat-soluble pigments from *Porphyra nereocystis*. The characteristic absorption curve of chlorophyll *a* is apparent (small amounts of chlorophyll *d* may also be present). In these extracts the carotenoids absorb a large proportion of the light in the region 460 to 500 mμ.

major photosynthetic pigment. Indeed, it falls away to almost its lowest values in the regions where chlorophyll (and carotenoids) are absorbing best. Only slight shoulders remain at 660 and at 440 mμ to suggest a slight participation of chlorophyll. (The deviation in the region of 495 mμ between action and absorption may represent inactive absorption by carotenoids, *cf.* Fig. 14.)

Absorption and action spectra for *P. naiadum* are given in Fig. 15. In its general features the activity curve resembles that obtained for *P. nereocystis*; that is, high photosynthetic activity in the regions of strong phycobilin ab-

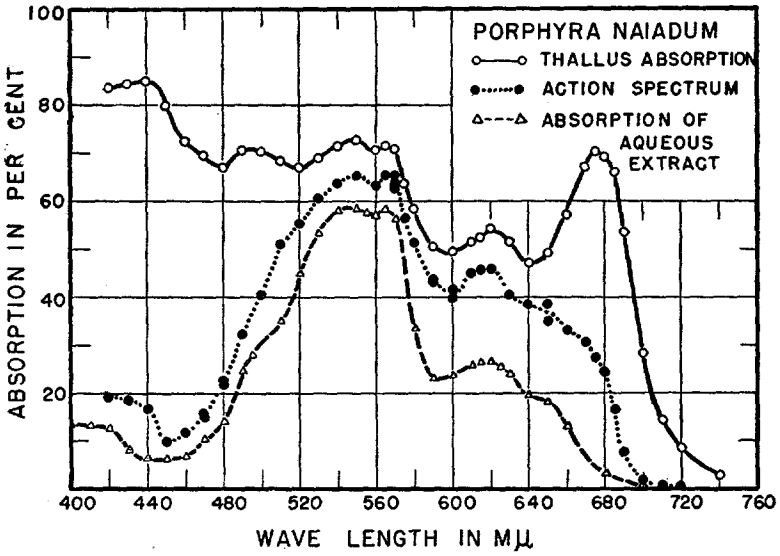

FIG. 15. Absorption and action spectra of *Porphyra naiadum*. This species contains more phycocyanin than *P. nereocystis*; this is reflected in the higher absorption in the orange (600 to 640 mμ) both in the tissue and in the water extract. The close over-all correspondence in the shape of the action spectrum and the water extract implicates in a striking manner the phycobilins as sensitizers in the reaction. Chlorophyll and carotenoids appear largely ineffective.

sorption and low activity in the chlorophyll-carotenoid regions. It is of further interest that the finer differences in the two curves can be correlated with variations in the phycobilins. (The detailed shape of each activity curve is significant, having been verified at least three times for each of these two species.) The increased photosynthetic rate at 620 mμ parallels a greater content of phycocyanin, as shown by the higher absorption in the region 600 to 640 mμ. Furthermore, the absence of a peak at 495 mμ in the activity curve of *P. naiadum* is duplicated in the absorption curve of the phycobilin extract (easily obtained by placing this species in distilled water). The close agreement

in shape of the activity curve and the water extract extends throughout the spectrum and includes the peak at 420 mμ. The absolute height of the phycocyanin peak is somewhat lower in the extract, a situation which may find explanation in the fact that this pigment is more unstable than phycoerythrin and does not extract as readily.

The tendency toward participation of phycocyanin in photosynthesis is seen still more in *P. perforata*. In the normal slate-grey (or greenish) thallus shown in Fig. 16, the phycocyanin "hump" becomes the most conspicuous part of the

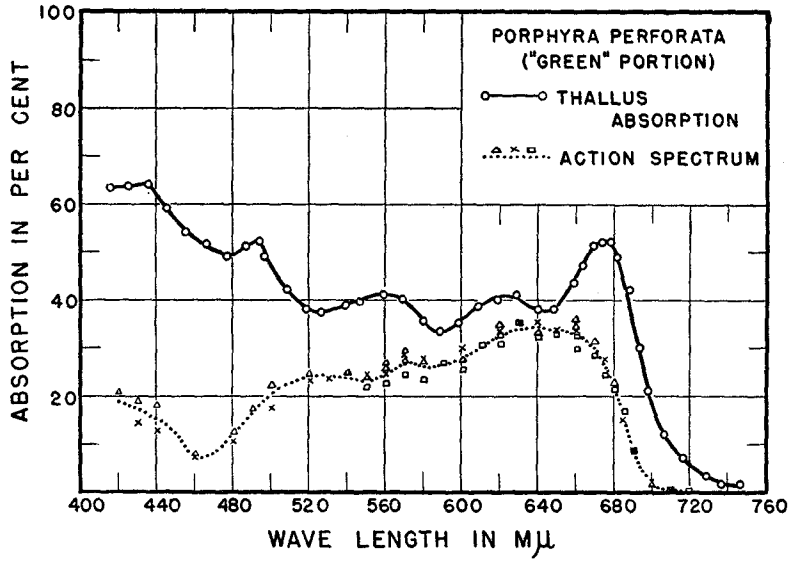

FIG. 16. Absorption and action spectra of *Porphyra perforata* (slate-green vegetative portion). The broad phycocyanin maximum (600 to 650 mμ) is the region of highest photosynthetic activity although phycoerythrin is also active. (The data were taken from several experiments using different thalli.)

action spectrum, being higher here than in the phycoerythrin region. The action spectrum falls off steeply toward the regions of chlorophyll (and carotenoid) maximal absorption. The slight rise toward 420 to 430 mμ may well correspond to increased phycobilin absorption (*cf.* Fig. 15) rather than to that of chlorophyll. Fig. 17 shows the action spectrum (more roughly taken) of the red-brown thallus margins of the same alga. Here the higher content of phycoerythrin brings about a corresponding change in the shape of the activity curve, now at its maximum in the region of phycoerythrin absorption.

Closely related to these porphyras is a rarer alga, *Porphyrella gardneri*. The deep red blades of this alga are delicate and very thin (somewhat over 10 to 15μ in thickness); it gave the most rapid electrode response of any alga tested.

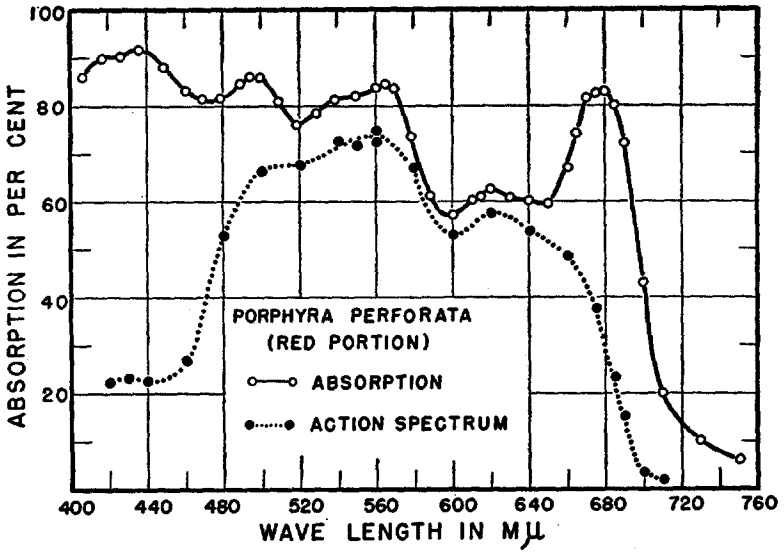

FIG. 17. Absorption and action spectra of *Porphyra perforata* (the red carposporic margins). The total absorption is higher here than in the paler vegetative portions of the thallus, also absorption by phycoerythrin exceeds that by phycocyanin. The photosynthesis curve reflects this change in phycobilins and is maximal in the phycoerythrin region. Again, chlorophyll (and carotenoids) appear largely inactive.

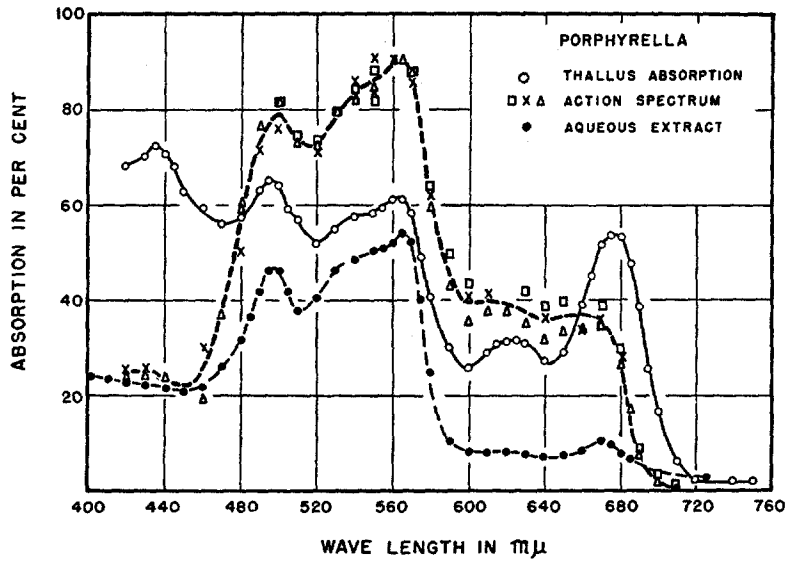

FIG. 18. Absorption and action spectra of *Porphyrella gardneri*. The activity curve is based upon data from three independent determinations using different thalli, but all corrected in accordance with the thallus absorption curve given above (the triangles refer to photosynthesis data taken with this thallus) and the relative rates set equal to 90 at 560 mμ. The crude water extract contains phycoerythrin along with protein impurities that contribute to general scattering. Also present is a trace of chlorophyll (indicated by the rise at 670 mμ) that remained after treatment of the extract with ether. Phycocyanin if originally present has not persisted to any extent in this extract.

Absorption and action spectra for *Porphyrella* given in Fig. 18 show a detailed similarity to *P. nereocystis* discussed above. The peak of the activity curve has been set above thallus absorption, in order to emphasize that absolute rates have not been measured and that the plotting is done in an arbitrary way. From the shape of the activity curve it is clear that light absorbed by the phycobilins (predominantly phycoerythrin here) is much more efficiently utilized than that absorbed by chlorophyll or carotenoids.

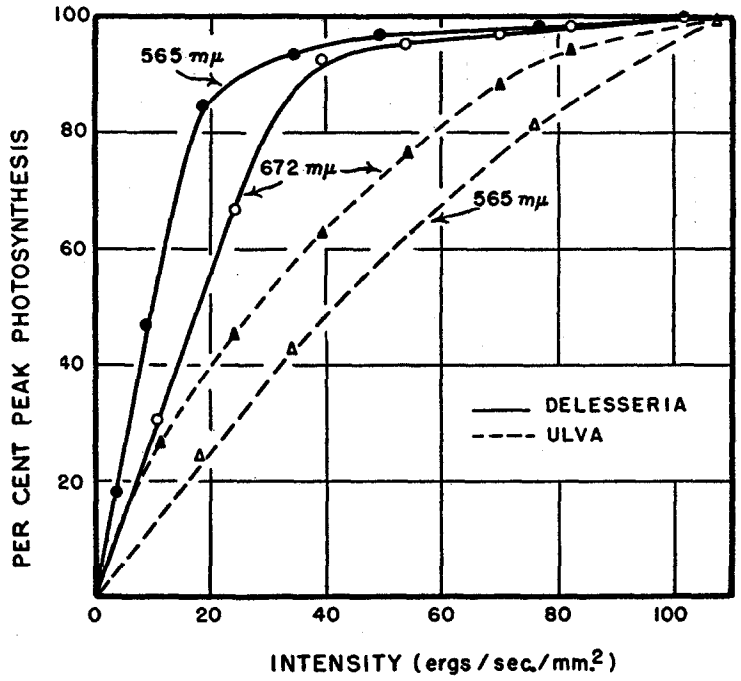

FIG. 19. Comparison of photosynthesis and light intensity in *Delesseria* and *Ulva*. Incident intensities are given in absolute units and photosynthesis in arbitrary units, the highest rate of photosynthesis being set to 100 in each case. The rather narrow bands of green and red light (centered at 565 and 675 mμ) were isolated from the monochromator using a wide exit slit. Photosynthesis was measured in the apparatus shown in Fig. 1 while a continuous flow of sea water was maintained.

(*Florideae*).—These, the higher red algae, are often thicker, but several usable species were available. The lateral blades of *Delesseria decipiens* are one cell thick, and have perhaps the clearest red color of all those studied. They were collected from shaded rock faces or cave entrances and are representative of the "shade" form of red algae. In contrast to *Porphyra naiadum* and *Ulva*, photosynthesis in *Delesseria* is light-saturated at relatively low intensities (30 to 50 ergs/sec./mm.^{2}), as is shown in Fig. 19. Limiting CO_2

concentrations may be responsible for the gradual falling off in rate shown by *Ulva* but apparently not for the abrupt leveling off in *Delesseria*, since the maximum rate of oxygen evolution was higher for *Ulva*. In *Delesseria* saturation in green and red light was at the same level of oxygen production, perhaps suggesting a common pathway of utilization for light absorbed by different pigments; *i.e.*, phycoerythrin and chlorophyll.

The absorption and action spectra for *Delesseria* shown in Fig. 20 are generally similar to those obtained for the redder species of Bangiales. There is

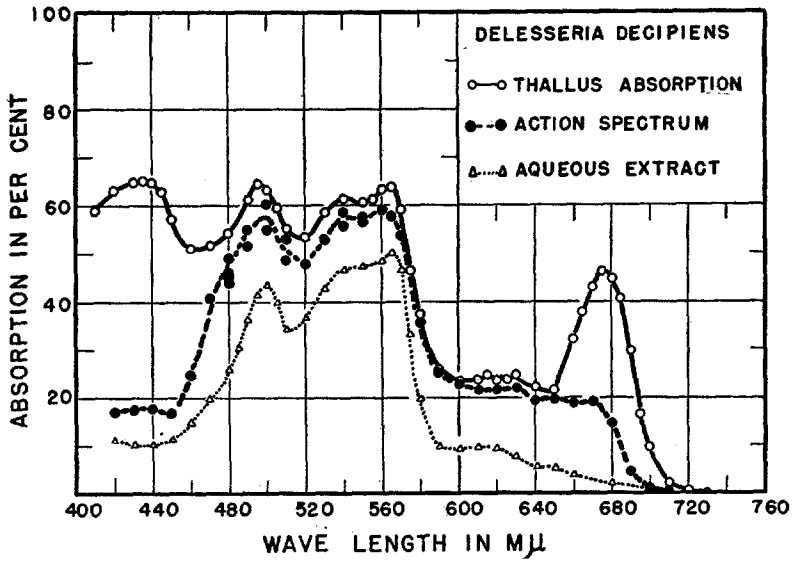

FIG. 20. Absorption and action spectra of *Delesseria decipiens*. This is a very red thallus, with a minimum of phycocyanin, and a somewhat low chlorophyll peak in the red. The action spectrum rather closely parallels the absorption of the aqueous extract (largely phycoerythrin).

little phycocyanin present, as is shown by the slight maximum at 620 mμ, but all three of the phycoerythrin peaks are well reflected in the action spectrum. On the other hand, the chlorophyll absorption peaks may be represented only by the faintest suggestion of shoulders or "foothills" in the activity curves.

Schizymenia pacifica and *Myriogramme spectabilis*, two rather deep growing algae with bright red thalli, many cells thick, gave very similar absorption and action spectra (Figs. 21 and 22). Detailed action spectra, uncorrected for back reflection, were also determined for *Antithamnion uncinatum* (a filamentous alga, collected from the stipes of *Nereocystis* some 8 meters down from the pneumatocyst) and *Cryptonemia ovalifolia*, both being approximately cherry

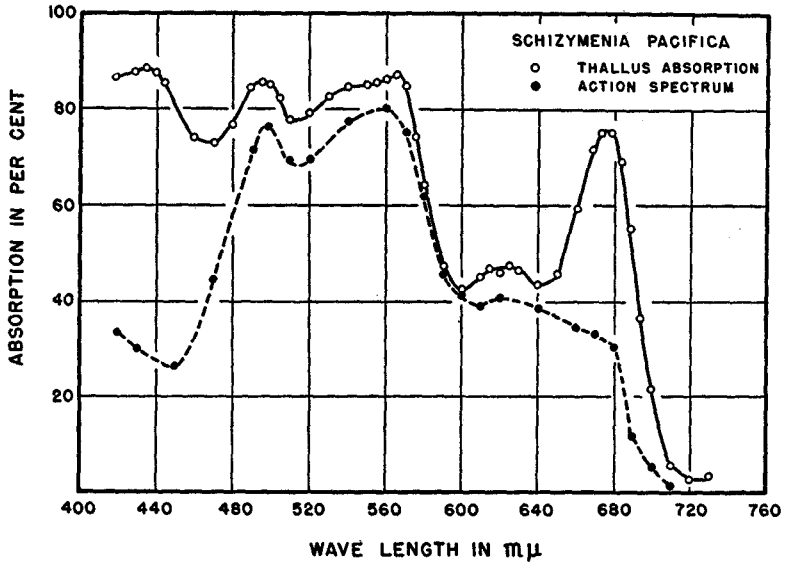

FIG. 21. Absorption and action spectra of *Schizymenia pacifica*. The absorption curve of this rather thick, cherry-red thallus is considerably higher than in *Delesseria*. The shapes of the activity curves are, however, much the same.

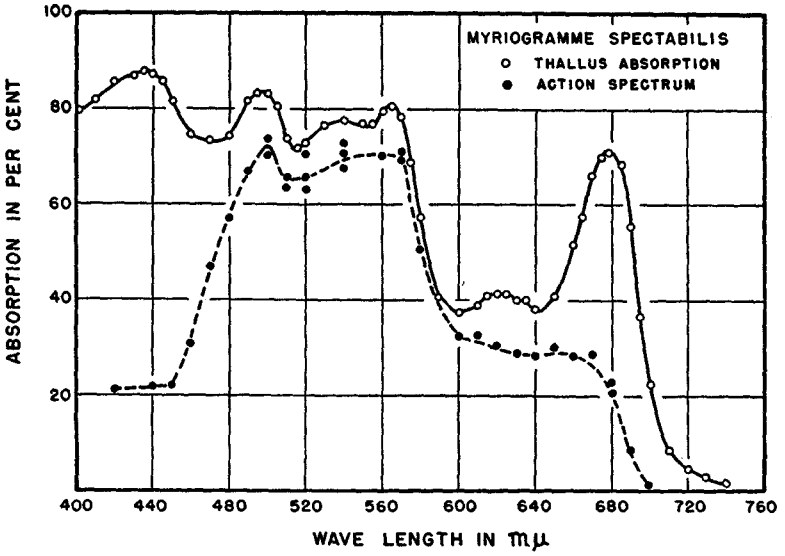

FIG. 22. Absorption and action spectra of *Myriogramme spectabilis*. This bright rose-red alga was found growing epiphytically on *Nereocystis*, some 8 meters below the pneumatocyst.

red in color. All these algae showed a "phycoerythrin type" activity curve with low activity in the regions of maximum chlorophyll absorption.

Blue-Green Algae (Cyanophyta).—

The results obtained from preliminary studies of a few marine filamentous blue-green algae such as *Anabaena* and *Oscillatoria* differ from those obtained by Emerson and Lewis (1942) for *Chroococcus* in showing less activity in the regions of chlorophyll absorption. The activity curves are rather like those of *Porphyra perforata*. Conditions of culture, especially light intensity (*cf.* Harder, 1923), may be responsible for this result which will be described elsewhere.

DISCUSSION

The striking result of these investigations is not so much the participation of phycoerythrin and phycocyanin in photosynthesis of red algae (this had been fairly clear before) but rather the relative *inactivity* of chlorophyll and carotenoids. Apparently light absorbed by the latter pigments is only very slightly used in the process of oxygen production. Expressed in another way, light absorbed by the phycobilin pigments (phycoerythrin and phycocyanin) is utilized with good efficiency, while that absorbed by chlorophyll (and carotenoids) is utilized with poor efficiency, if at all. The detailed action spectra correspond, almost point for point, with the absorption spectra of these chromoproteins, while dropping to very low values in the spectral regions where the lipoid-soluble pigments absorb most strongly. Chlorophyll and carotenoids seem to be only slightly active in a photochemical sense, the primary active absorbing substances being phycoerythrin and phycocyanin.

This situation is so unusual that question may arise as to the soundness of the method employed. Internal evidence of the latter is at hand in the very "regular" action spectra of the green and brown algae (Figs. 8 and 11); Levring's results also roughly parallel our red algal findings, though not in detail. There was, however, still the possibility that some unknown characteristic of the electrode method was picking up the phycoerythrin action spectrum, while neglecting the chlorophyll-carotenoid one. Other methods were therefore employed in this laboratory to check the findings. These have included the Warburg, Winkler, Fenn, and Cartesian diver methods for oxygen production (the latter two apparently finding here their first application to photosynthetic measurements). These will be reported in detail elsewhere, with special emphasis on absolute rates and quantum yields. It may be said, however, that all these methods showed very good agreement with the electrode findings at enough critical wave lengths that the detailed action spectra could be presented with confidence.

At the present time it is not possible to make definite statements concerning the path of energy absorbed by phycoerythrin. In view of the poor utilization

of light absorbed directly by chlorophyll, the transfer of energy from phycoerythrin to chlorophyll seems unlikely, since the latter process would have to be more efficient than the former. A study of the fluorescence spectra obtained with living red algae when excited by light absorbed primarily by phycoerythrin or chlorophyll should answer the question as to an energy transfer and, furthermore, give insight into direct photochemical activity on the part of chlorophyll. (A high fluorescence yield might be expected for energy absorbed directly by chlorophyll, unless the absorbed energy can be dissipated in other ways.) Van Norman, French, and MacDowall (1948) have made studies in this direction using *Gigartina* and *Iridaea*, but were unable to form definite conclusions as to whether energy transfer was involved. They obtained rather complex fluorescence spectra with three peaks, probably due to phycoerythrin, phycocyanin, and chlorophyll, although identification was uncertain.

The poor participation of chlorophyll is the more remarkable because it is present to about the same concentration in the red algae as in the green and brown algae (Seybold and Engle, 1938). True, this is largely chlorophyll *a*, chlorophyll *b* being replaced by chlorophyll *d* (Manning and Strain, 1943). Possibly chlorophyll *b* or *c* must be present for proper functioning of the chlorophyll system. Chlorophyll is perhaps biochemically or topographically unavailable to the necessary enzyme systems of the red algae; it may lie deep within the plastid, while phycoerythrin may be upon the surface. The ease of phycoerythrin's detachment from the plastid, and the very red color of the latter suggest this. (Optical shading of one pigment by the other is probably not the cause, because phycoerythrin transmits light very well in the regions where chlorophyll absorbs, and *vice versa*.) It is even possible that the protein of the phycoerythrin is itself an enzyme of photosynthesis which, because it is very firmly attached to its chromophore (see Lemberg, 1928), is unable to couple with chlorophyll.

But there still remains the puzzle as to why chlorophyll is present in such quantity if it is not photochemically active in the red algae. It may of course still participate in the "dark" reactions. There is some evidence that the chlorophyll in red algae may be activated in some cases. One species of *Iridophycus* occurs on the Pacific Coast which bleaches to an almost green color at higher tide levels. Rough action spectra of such plants showed distinct peaks corresponding to chlorophyll absorption, though the residual phycoerythrin was still effective. Some of the curves presented in this paper (Figs. 18 and 20) show a slight hint of this effect. Further work is being done on this alga with the hope of throwing light on the role of chlorophyll in red algae.

A few words should be added here about the bearing of this investigation on the ecology of marine algae and the question of complementary chromatic adaptation (*cf.*, Rabinowitch, 1945; Levring, 1947; Strain, 1949). In littoral waters the red and blue ends of the sun's spectrum are selectively absorbed

so that at depths below several meters algae are exposed primarily to green and blue-green light of diminishing intensity. The red algae in particular with their high content of phycoerythrin are admirably equipped for this environment since they absorb such light strongly and utilize it efficiently. On the other hand, inactivity of chlorophyll would be no great handicap, since the available light field does not contain wave lengths absorbed by this pigment. If spectral assimilation curves are plotted for *Delesseria* at different depths, as Levring has done, one finds that at 10 meters almost all the photosynthetic activity is limited to the region 470 to 590 mμ. (These curves involve consideration at each wave length of three factors: intensity of incident light, thallus absorption, and relative efficiency of utilization of absorbed light.) It is unlikely that chlorophyll *d*, which may be mainly responsible for absorption beyond 690 mμ, adds significantly to the photosynthetic capacity, even of surface-growing red algae:—a possibility discussed by Manning and Strain (1943). All the red algae tested by us showed almost no photosynthetic activity in this spectral region even though light absorption was still appreciable in some cases. (The chlorophyll *d* content of our algae was not determined.)

In view of the photosynthetic effectiveness of the phycobilins it would seem logical to assume that the present vertical distribution of red algae has been influenced, at least in part, by this capacity and that some species have been thus enabled to extend their distribution to depths not readily penetrated by other algae. The fact that other algae may also be found at great depths or that red algae are also to be found in shallower waters in no way alters this possibility. Obviously the distribution of algae is influenced by many factors each of which must be considered. Shade plants such as *Delesseria* (Fig. 19) may be restricted to depths or shaded rocks because they cannot withstand higher light intensities. Other factors such as increased chlorophyll content or decreased respiratory rate may permit algae lacking suitable accessory pigments to remain above the compensation point at greater depths.

In general it may be concluded that while chlorophyll is the major photosynthetic pigment of most plants, other pigments can also participate, and in some cases (red algae) take over a major role as effective light absorbers.

SUMMARY

A polarographic oxygen determination, with tissue in direct contact with a stationary platinum electrode, has been used to measure the photosynthetic response of marine algae. These were exposed to monochromatic light, of equal energy, at some 35 points through the visible spectrum (derived from a monochromator). *Ulva* and *Monostroma* (green algae) show action spectra which correspond very closely to their absorption spectra. *Coilodesme* (a brown alga) shows almost as good correspondence, including the spectral region absorbed by the carotenoid, fucoxanthin. In green and brown algae, light ab-

sorbed by both chlorophyll and carotenoids seems photosynthetically effective, although some inactive absorption by carotenoids is indicated.

Action spectra for a wide variety of red algae, however, show marked deviations from their corresponding absorption spectra. The photosynthetic rates are high in the spectral regions absorbed by the water-soluble "phycobilin" pigments (phycoerythrin and phycocyanin), while the light absorbed by chlorophyll and carotenoids is poorly utilized for oxygen production. In red algae containing chiefly phycoerythrin, the action spectrum closely resembles that of the water-extracted pigment, with peaks corresponding to its absorption maxima (495, 540, and 565 mμ). Such algae include *Delesseria*, *Schizymenia*, and *Porphyrella*. In the genus *Porphyra*, there is a series *P. nereocystis*, *P. naiadum*, and *P. perforata*, with increasingly more phycocyanin and less phycoerythrin: the action spectra reflect this, with increasing activity in the orange-red region (600 to 640 mμ) where phycocyanin absorbs.

In all these red algae, photosynthesis is almost minimal at 435 mμ and 675 mμ, where chlorophyll shows maximum absorption. Although the chlorophylls (and carotenoids) are present in quantities comparable to the green algae, their function is apparently not that of a primary light absorber; this role is taken over by the phycobilins. In this respect the red algae (Rhodophyta) appear unique among photosynthetic plants.

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